

Yaws Disease Caused by *Treponema pallidum* subspecies *pertenue* in Wild Chimpanzee, Guinea, 2019

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Yaws-like lesions are widely reported in wild African great apes, yet the causative agent has not been confirmed in affected animals. We describe yaws-like lesions in a wild chimpanzee in Guinea for which we demonstrate infection with *Treponema pallidum* subsp. *pertenue*. Assessing the conservation implications of this pathogen requires further research.

Several monkey species in sub-Saharan Africa are infected with *Treponema pallidum* subspecies *pertenue* (TPE) and typically manifest yaws-like lesions on the face and distal extremities or syphilis-like lesions in the anogenital region (1). Reports of nonhuman primates (NHPs) infected with TPE came from West Africa in the 1960s. These studies were based on seroprevalence studies finding that yellow baboons (*Papio cynocephalus cynocephalus*) had a 60% seroprevalence rate for treponemal-specific antibodies (2,3). Whole-genome sequencing of the isolate collected from these baboons later revealed similarities with TPE causing yaws in humans (3,4). In the late 1980s in Gombe National Park in Tanzania, olive baboons (*Papio anubis*) with genital ulcerations were found to have yaws-like infections of the skin (5). Later genetic and serologic studies confirmed

infections with *T. pallidum* in olive baboons at many sites in Tanzania (6,7).

Both genital and orofacial lesions attributable to TPE infection have been documented in several NHP species across sub-Saharan Africa (African green monkeys [*Chlorocebus sabaeus*] in Bijilo Forest Park, The Gambia, and Niokola-Koba National Park, Senegal; sooty mangabeys [*Cercocebus atys atys*] in Taï National Park, Côte d'Ivoire) (1; B. Mubemba et al., unpub. data, <https://doi.org/10.1101/848382>). Two studies observed that TPE infections remain geographically widespread in Tanzania and affect olive baboons, yellow baboons, vervet monkeys (*Chlorocebus pygerythrus*), and blue monkeys (*Cercopithecus mitis*), as well as grivet monkeys (*Chlorocebus aethiops*) from Ethiopia (8,9).

Symptoms and skeletal deformation have also been observed in great apes, specifically gorillas (*Gorilla gorilla*) in the Republic of Congo, Gabon, and Cameroon (10), as well as in chimpanzees (*Pan troglodytes*) in Cameroon, Uganda, and Côte d'Ivoire, and are suggestive of TPE infections (10; F.H. Leendertz, pers. comm., 2019 Nov 1), but matching diagnostics are currently unavailable. The only diagnostic evidence is based on TPE DNA from 2 chimpanzee (*P. troglodytes verus*) bones (11) and gorilla feces (9) of unknown individual great apes, so no link between diagnostics and clinical signs can be made. We present matching clinical and molecular evidence of TPE infection in a wild great ape.

The Study

We found a cachectic wild adult female chimpanzee (*Pan troglodytes verus*) with severe yaws-like lesions on the mouth and lips in a mining concession in Sangaredi area, Guinea (Figure 1, panel A). The chimpanzee

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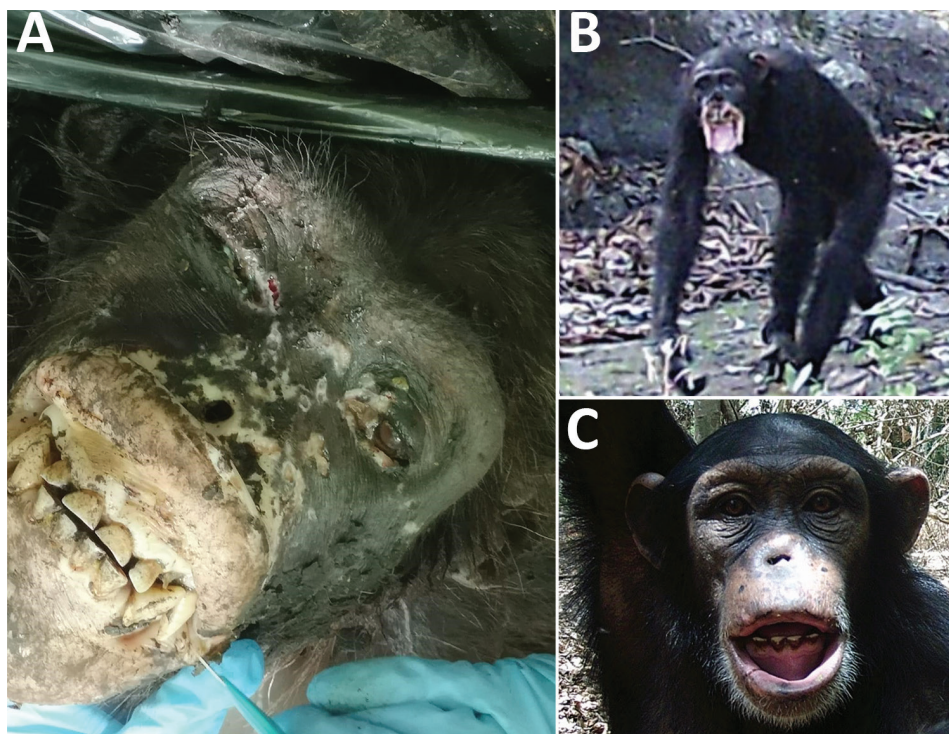


Figure 1. Yaws-like lesions in wild chimpanzees, Guinea. A) Yaws-like lesions observed during a necropsy of an adult female chimpanzee found in the Sangaredi area, Guinea. B, C) Camera trap images showing yaws-like lesions on adult (B) and juvenile (C) chimpanzees in Haut Niger National Park, Guinea.

was in visible agony and had to be euthanized; we performed a necropsy on the body. Gross pathology of the skin revealed a marked depigmentation on hypertrophied edematous lips; crusts and ulcers were present on the head, and much of the nose was missing. The eyes were shrunk and purulent and surrounded by crusts, and the corneas were opaque. We preserved samples of lesioned skin in 10% formalin and RNAlater (Thermo Fisher, <https://www.thermofisher.com>).

We analyzed formalin-fixed skin samples with both histological and immunohistochemical methods, as previously described (6). Histopathological features of the skin biopsies were compatible with treponemal infection (Figure 2, panel A). Skin lesions were characterized by irregular epidermal proliferation of different extents. The epidermis developed hyperkeratosis and hypertrophy of the epidermal rete pegs, which branched and projected deeply into the corium. Admixed areas with severe superficial erosions or deep ulcerations were visible. A moderate to severe mixed cell infiltration composed of lymphocytes and histiocytes was present in the underlying dermal layer. The cellular reaction was most pronounced around the dermal blood vessels and hair follicles, resulting in superficial and deep perivascular dermatitis. The epidermal surface was covered with a dried serosanguineous discharge. Immunohistochemical analyses failed to visualize treponemes,

which is a frequent problem resulting from low numbers of bacteria at lesion sites (6).

We extracted DNA from 2 facial lesion biopsies stored in RNAlater and performed molecular investigations (Appendix, <https://wwwnc.cdc.gov/EID/article/26/6/19-1713-App1.pdf>). High-throughput sequencing analysis resulted in a 24-fold average coverage of the TPE genome; 98.6% of the genome was covered by ≥ 1 read and 97.6% by ≥ 3 reads. Bayesian Markov chain Monte Carlo analysis of a genomic alignment comprising this reconstructed TPE genome, all other available TPE and *Treponema pallidum* subsp. *endemicum* (TEN, bejel) genomes, and a selection of *Treponema pallidum* subsp. *pallidum* (TPA, syphilis) genomes available in Genbank (Appendix Table) revealed that the chimpanzee-derived genome clustered within the well-supported TPE clade, indicating that TPE is responsible for the clinical picture observed in this particular wild chimpanzee (Figure 2, panel B). More precisely, this new chimpanzee-derived genome belongs to a clade consisting of TPE strains isolated from NHPs in far western Africa in Gambia, Guinea Bissau, Senegal, and Guinea, in agreement with recent observations that genomic diversity of TPE strains infecting NHPs appears to be geographically structured (9; B. Mubemba et al., unpub. data, <https://doi.org/10.1101/848382>). Yaws is principally a skin disease, and it seems likely that the poor condition of this animal was caused by another

unknown but likely traumatic cause, perhaps coupled with associated secondary infections, although our field necropsy was not able to identify an alternative cause of her cachectic condition.

To determine whether TPE might affect other chimpanzees in Guinea, we examined videos collected

by camera traps set near the Chimpanzee Conservation Center in Haut Niger National Park. During 2018–2019, in 10 different camera trap locations, we observed 12 chimpanzees (1 juvenile, 3 subadults, and 8 adults) with severe lesions. The lesions observed in these images closely resembled those of the wild female from

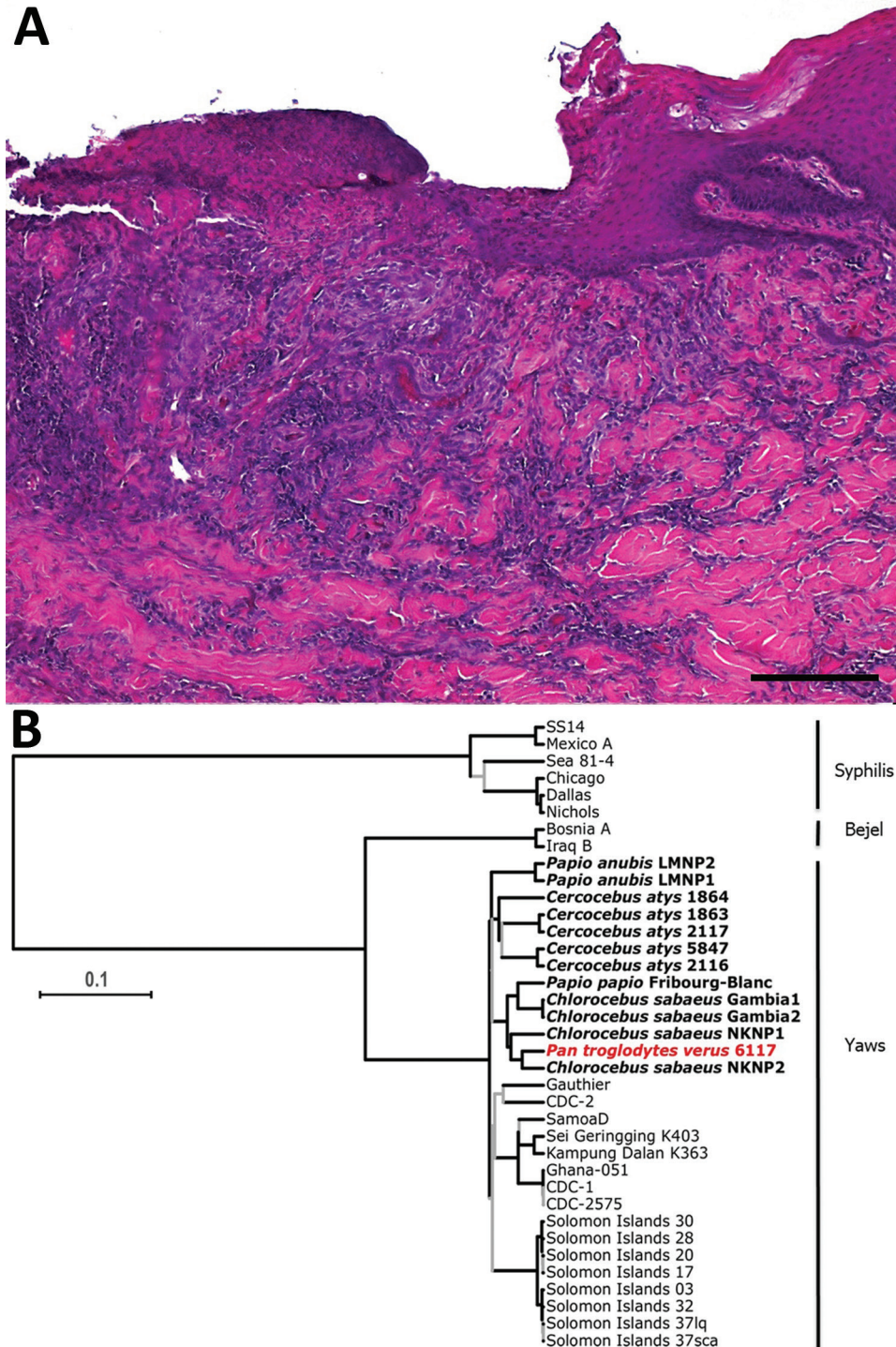


Figure 2. Histopathologic analysis of yaws-like lesions in a wild chimpanzee, Guinea, and phylogenetic placement of the *Treponema pallidum* subspecies *pertenue* strain. A) Histopathologic evidence suggestive of a treponemal infection. Shown here is superficial ulcerative pyogranulomatous dermatitis including formation of a mixed inflammatory cell infiltration, predominantly neutrophil granulocytes. Deeper dermal layers show the formation of a perivascular lymphocytic inflammatory cell infiltrate, focal folliculitis, and perifolliculitis. Skin areas adjacent to ulcerated parts show irregular epidermal hyperplasia, consistent with treponemal infections. The ulcerated areas were covered by a serocellular crust. Scale bar indicates 200 μ m. B) Maximum clade credibility tree of *T. pallidum* strain genomes. Red indicates the chimpanzee genome generated in this study. All simian-infecting strains are shown in bold with labels showing the species of nonhuman primate, and the diseases caused by each type of bacteria are shown at right. Branches supported by posterior probabilities <0.95 in the Bayesian Markov chain Monte Carlo tree are indicated in gray. Scale bar indicates nucleotide substitutions per variable site.

the Sangaredi region described in this article, including shrunken eyes, deformation of the face, absence of the nose, and hypertrophied and depigmented lips (in 1 case, the lips were completely missing; Figure 1, panels B, C). Molecular investigations of the pathogen(s) causing these infections is clearly warranted, perhaps through noninvasive screening of TPE in feces, bones, or primate-associated flies (9,12).

Conclusions

This study links yaws-like pathology to the actual detection of TPE in a wild chimpanzee, providing evidence that at least part of the suggestive lesions often observed in wild great apes are caused by this pathogen. These data join a growing body of evidence demonstrating that many NHP species across sub-Saharan Africa are infected with TPE (1,9). This finding could potentially be problematic for the ongoing campaign to eradicate TPE globally by 2030 (13), although, clearly, data from TPE-infected humans in this region are needed to determine whether zoonotic transmission of this pathogen occurs. Given the severity of lesions, it is evident that individual animal fitness is affected. The impact of this disease on NHP populations is unknown but could be assessed through long-term monitoring.

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Appendix

Molecular and Bioinformatics Analyses

We extracted DNA from 2 chimpanzee facial lesion biopsies using the DNeasy blood and tissue extraction kit (QIAGEN, <https://www.qiagen.com>) following the manufacturer's protocol. We screened samples with a quantitative PCR targeting the *polA* gene of *Treponema pallidum*, as described previously (1); both samples were positive. We converted DNA extracts into dual indexed Illumina libraries using the NEBNext Ultra II DNA Library Prep kit (New England Biolabs, <https://www.neb.com>). We enriched the libraries for TPE through in-solution hybridization capture, as previously described (2; B. Mubemba et al., unpub. data, <https://doi.org/10.1101/848382>) sequenced on an Illumina NextSeq (v2 chemistry, 2x150 cycles; <https://www.illumina.com>). We quality-filtered the reads using Trimmomatic v0.38 (removing leading and trailing reads <Q30; clipping reads where average base quality across 4 bp was <30; removing surviving reads <30 nt long) (3). We merged surviving read pairs with Clip and Merge v1.7.8 (<https://anaconda.org/bioconda/clipandmerge>). We combined merged reads and surviving singletons and mapped them to TPE Fribourg-Blanc (RefSeq ID NC_021179.1) using BWA-MEM with a minimum seed length of 29. We sorted mapped reads using Picard's SortSam, de-duplicated them with Picard's MarkDuplicates (<https://broadinstitute.github.io/picard/index.html>), and removed alignments with MAPQ <30 and a mapping length <30 nt using SAMtools (4). Finally, we merged all mapped reads of individual library samples to produce single TPE draft genome. We used Geneious v.11 to call a consensus genome requiring a minimum of 3 unique reads to cover a position for it to be called and applying a majority consensus rule (5).

We performed whole genome alignment using the multiple sequence alignment program MAFFT (6). We then removed all putative recombinant genes (2) and selected conserved blocks using the Gblocks tool (7) in SeaView v4 (8). We performed Bayesian Markov chain Monte Carlo phylogenomic analysis in BEAST (version 1.10.4; <https://www.mybiosoftware.com>) on the resulting alignment of 4,213 variable positions (after stripping off of all ambiguities and identical sites in the final dataset); settings of the analysis were a strict clock model and a coalescent process assuming constant population size. We examined the output of multiple chains of 10,000,000 generations for convergence and appropriate sampling of the posterior using Tracer (version 1.7.1) (9) before merging tree files using Log Combiner (version 1.10.4) (10). We picked the best representative tree from the posterior set of trees and annotated it with Tree Annotator (version 1.10.4: distributed with BEAST). We further edited the resultant maximum clade credibility (MCC) tree file using iTOL (<https://itol.embl.de>) (11). *T. pallidum* sequences used in this study are listed in the Appendix Table (12–24).

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Appendix Table. Published *Treponema pallidum* (TP) sequences used in the study of *T. pallidum* in wild chimpanzees.

Isolate ID	GenBank accession no.	Host	TP spectrum	Country	Reference
Bosnia A	CP007548.1	<i>Homo sapiens</i>	Bejel	Bosnia	(12)
Iraq_B	CP032303.1	<i>Homo sapiens</i>	Bejel	Iraq	(13)
Nichols	NC_021490.2	<i>Homo sapiens</i>	Syphilis	USA	(14)
SS14	NC_021508.1	<i>Homo sapiens</i>	Syphilis	USA	(14)
Chicago	NC_017268.1	<i>Homo sapiens</i>	Syphilis	USA	(15)
Mexico A	NC_018722.1	<i>Homo sapiens</i>	Syphilis	Mexico	(14)
Dallas	NC_016844.1	<i>Homo sapiens</i>	Syphilis	USA	(16)
Seattle 81–4	CP003679.1	<i>Homo sapiens</i>	Syphilis	USA	(17)
Fribourg-Blanc	NC_021179.1	<i>Papio papio</i>	Yaws	Guinea	(18)
Samoa D	NC_016842.1	<i>Homo sapiens</i>	Yaws	Samoa	(19)
Gauthier	NC_016843.1	<i>Homo sapiens</i>	Yaws	Republic of the Congo	(19)
CDC-1	CP024750.1	<i>Homo sapiens</i>	Yaws	Ghana	(20)
CDC-2	NC_016848.1	<i>Homo sapiens</i>	Yaws	Ghana	(19)
CDC_2575	CP020366	<i>Homo sapiens</i>	Yaws	Ghana	(21)
Ghana-051	CP020365	<i>Homo sapiens</i>	Yaws	Ghana	(21)
Kampung_Dalan_K363	CP024088.1	<i>Homo sapiens</i>	Yaws	Indonesia	(22)
Sei_Geringging_K403	CP024089.1	<i>Homo sapiens</i>	Yaws	Indonesia	(22)
Solomon Islands 03	ERR1470343	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 17	ERR1470344	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 20	ERR1470335	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 28	ERR1470338	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 30	ERR1470334	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 32	ERR1470342	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 37 liq	ERR1470330	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Solomon Islands 37 sca	ERR1470331	<i>Homo sapiens</i>	Yaws	Solomon Islands	(23)
Gambia-1	<u>SRR4308597</u>	<i>Chlorocebus sabeus</i>	Yaws	Gambia	(24)
Gambia-2	SRR4308605	<i>Chlorocebus sabaeus</i>	Yaws	Gambia	(24)
Senegal NKNP-1	<u>SRR4308606</u>	<i>Chlorocebus sabaeus</i>	Yaws	Senegal	(24)
Senegal NKNP-2	<u>SRR4308607</u>	<i>Chlorocebus sabaeus</i>	Yaws	Senegal	(24)
LMNP-1	CP021113.1	<i>Papio anubis</i>	Yaws	Tanzania	(24)
LMNP-2_BS5	<u>SRR4308598</u>	<i>Papio anubis</i>	Yaws	Tanzania	(24)
LMNP-2_BS6	<u>SRR4308599</u>	<i>Papio anubis</i>	Yaws	Tanzania	(24)
LMNP-2_BS7	<u>SRR4308601</u>	<i>Papio anubis</i>	Yaws	Tanzania	(24)
LMNP-2_BS8	SRR4308602	<i>Papio anubis</i>	Yaws	Tanzania	(24)
1863-Hato	SRR4308604	<i>Cercocebus atys</i>	Yaws	Côte d'Ivoire	(24)
1864-IGU	SRR4308596	<i>Cercocebus atys</i>	Yaws	Côte d'Ivoire	(24)
2117-BAK	SAMN13258074	<i>Cercocebus atys</i>	Yaws	Côte d'Ivoire	Mubemba et al.*
2116-OKA	SAMN13258075	<i>Cercocebus atys</i>	Yaws	Côte d'Ivoire	Mubemba et al.*
5847-CHAT	SAMN13258076	<i>Cercocebus atys</i>	Yaws	Côte d'Ivoire	Mubemba et al.*

*B. Mubemba et al., unpub. data, <https://doi.org/10.1101/848382>.